77 days-long toxicity study of Avemar (M1) in F344 rats and in C57BL/6 mice

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Aim of the study
To investigate the effects of the fermented wheat germ extract (M1) on the growth and histopathological status of laboratory animals.

Experimental design
13-13 male F344 rats and C57BL/6 mice animals were included into the experimental groups treated with the substance M1. In the experiments the same number of animals served as controls. The animals were ear-marked individually, and were carefully checked daily to note and register the eventual pathological signs and symptoms (fur, color of the nose and ears, body temperature). Changes in body weight were also registered. On the 77^{th} day from the beginning of the experiment all animals have been killed in anesthesia. Samples were taken for histological study from all organs and tissues following the relevant instruction of RITA – Registry of Industrial Toxicology Animal-data (Bahnemann R, Jacobs M, Karbe E,Kauffmann W, Morawietz G, Nolte T, Rittinghausen S: RITA – Registry of Industrial Toxicology Animal-data – Guides for organ sampling and trimming procedures in rats. Exp Toxic Pathol 47: 247-266, 1995). Histological preparations (hematoxylin eosin stained sections) were prepared from each organs and tissues of randomly selected 3-3 animals from each experimental groups. The slides were studied and evaluated by two certified pathologists in a blinded setting. All alterations differing from the normal histology of the respective organ/tissue were registered.

Treatment
3 g/kg body weight M1, in the form of 0.6 g/ml dilution, was administered per os, once daily, by stomach-tube. Vitamin C, 0.9 g/kg body weight/day, in the form of 0.18 g/ml solution, was also administered via gastric tube.

Histological studies were carried out in the organs and tissues of the following ear-marked 3-3 randomly selected animals from the treated groups:

Rats		Mice	
1. F344	II. (₣)	4. C57BL/6	IV. (¥)
2.	II. (Δ)	5.	IV. (€)
3.	II. (£)	6.	IV. (§)

Roman numbers are indicating the cage number, ear marks are indicating individual animals within the respective cage.

Results
There was no difference in the weight gains of the treated and untreated groups of animals. In Fig. 1 the growth curve of the F344 rats are shown. In Tables 1-4, the weight gains and the final organ weights of the treated animals are shown, respectively.

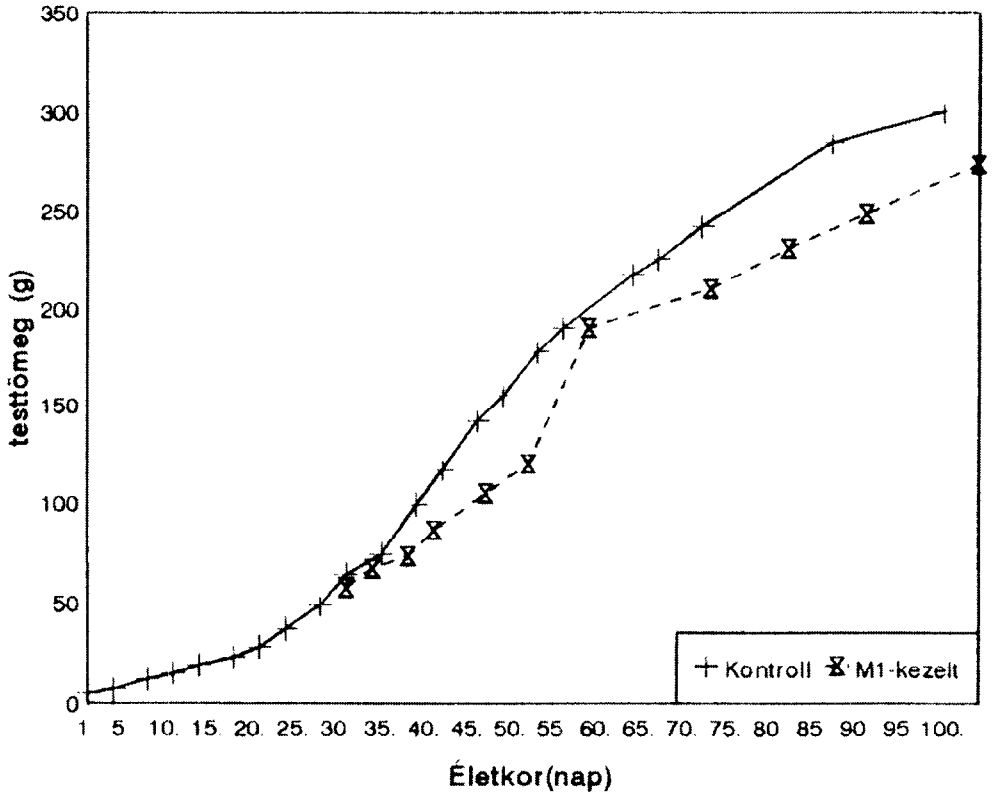

Table 1
F344 rats treated with substance M1 continuously. Changes in the body mass starting at the onset of the treatment and in different time points

Body mass	Time (days)										
	1.	4.	8.	11.	17.	22.	29.	40.	52.	61.	74.
Average	57.6	66.9	73.0	86.2	104.8	119.8	190.3	210.2	231.0	248.5	273.3
SD	11.2	11.0	10.2	10.6	13.2	16.0	24.2	27.0	25.4	30.8	21.1
SEM	3.2	3.2	2.9	3.1	3.8	4.6	7.0	7.8	7.3	8.9	6.1
Min.	42.0	48.0	57.5	68.6	82.7	90.0	131.0	146.0	174.0	170.0	234.7
Med.	57.0	69.2	73.7	89.1	109.6	126.0	196.5	217.5	237.0	254.0	271.6
Max.	76.0	82.5	87.4	97.7	121.5	141.0	213.0	236.0	260.0	284.0	307.4

Table 2
F344 rats treated with substance M1. Organ mass values on the 77th day of the treatment.

Organ mass	Mass (g)								
	Heart	Lung	Thymus	Spleen	Liver	kidney1	Kidney2	Testicle1	Testicle2
Average	0.86	1.9	0.26	0.63	13.1	1.18	1.17	1.46	1.41
SD	0.08	0.28	0.08	0.04	2.0	0.09	0.10	0.11	0.10
SEM	0.02	0.08	0.02	0.01	0.58	0.03	0.03	0.03	0.03
Min.	0.77	1.31	0.08	0.58	8.94	1.03	0.94	1.32	1.27
Med.	0.84	1.97	0.25	0.63	13.12	1.17	1.12	1.46	1.38
Max.	1.00	2.2	0.4	0.71	16.8	1.32	1.17	1.70	1.61

Table 3
C57BL/6 mice treated with substance M1 continuously. Changes in the body mass starting at the onset of the treatment and in different time points.

Body mass	Time (days)									
	1.	7.	13.	18.	32.	39.	48.	61.	67.	77.
Average	18.3	19.0	18.5	18.6	22.2	23.0	26.6	25.3	26.1	27.6
SD	2.7	3.2	2.5	2.5	2.3	2.6	2.4	2.2	2.2	2.4
SEM	0.8	1.02	0.8	0.8	0.8	0.9	0.8	0.7	0.7	0.8
Min.	14.6	14.8	15.3	15.5	17.8	18.9	23.2	21.6	22.8	23.2
Med.	18.4	18.7	18.3	17.5	22.9	23.4	26.8	25.5	26.1	28.3
Max.	23.0	24.5	23.0	23.6	25.6	26.7	30.5	28.3	29.4	31.3

Table 4
C57BL/6 mice treated with substance M1. Organ mass values on the 77th day of the treatment.

Organ mass	Mass (g)								
	Heart	Lung	Thymus	Spleen	Liver	Kidney1	Kidney2	Testicle1	Testicle2
Average	0.16	0.21	0.09	0.09	1.56	0.19	0.20	0.11	0.13
SD	0.02	0.03	0.01	0.01	0.15	0.02	0.02	0.02	0.01
SEM	0.005	0.01	0.004	0.004	0.05	0.007	0.005	0.005	0.004
Min.	0.14	0.16	0.07	0.07	1.27	0.15	0.18	0.09	0.11
Med.	0.17	0.21	0.09	0.10	1.57	0.18	0.19	0.11	0.13
Max.	0.19	0.25	0.11	0.11	1.75	0.22	0.23	0.15	0.15

Following the thorough histopathological examination of the organs of both the treated and control animals, no pathological alteration was found in either group of animals. The animals were randomly chosen for histopathology. The pathology experts were unaware of treatment allocation.

Conclusion
Chronic administration of the fermented wheat germ extract (M1) is safe in terms of weight gain and tissue histopathology studies in laboratory rats and mice.

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